CPU 238 is temporarily stored in an image line buffer 242 that enables the image to be displayed or otherwise recorded for later analysis.

FIGURE 10 illustrates a practical application of the above described systems for identifying a male cell 200 and a female cell 208 and for producing their corresponding scatter images 212 and 220. Male cell 200 includes a nucleus 202 that has been stained with a yellow fluorescent dye. In addition, a FISH probe 204 produces a fluorescent orange emission, indicating the presence of an X-chromosome in the nucleus, while a FISH probe 206 produces red fluorescence emission, indicating the presence of a Y-chromosome. Spectral decomposition of the fluorescence emissions from male cell 200, when the cell is illuminated with light from a green light source, results in a series of images on TDI detector 44, separated as a function of the wavelength of the light that is imaged. Green light that is incident on the cells has a narrow waveband, and image 212 of male cell 200 produced by green light scatter is only slightly convoluted by the spectral decomposition process. Green light scatter image 212 of cell 200 and its nucleus 202 appear on the left side of the TDI detector, while a fluorescent spot 214 corresponding to the yellow fluorescence emitted by nucleus 202 appears in the next few columns on the TDI detector. Furthermore, as a function of the different wavelengths of the fluorescence emitted by FISH probes 204 and 206, FISH spots 216 and 218 appear at locations spaced apart on the detector, but slightly blurred across the columns of TDI detector 44 due to the widths of their respective emission spectra. By analyzing the signals produced by the TDI detector, the FISH probes responsive to X and Y chromosomes are detected, enabling the user to determine that cell 200 is a male cell, since it includes both the X and Y chromosome. Similarly, female cell 208, when spectrally decomposed, also includes the characteristic yellow fluorescence of nucleus 210, but unlike the male cell, includes two FISH spots 216 corresponding to FISH probes 204, which indicates the presence of two X-chromosomes. Because TDI detector 44 also distinguishes the spatial position of male cell 200 and female cell 208, the corresponding spectral decompositions for these cells are readily separately resolved as both cells pass through the imaging system in the direction indicated by the arrow to the lower left of FIGURE 10. Again, it should be noted that a deconvolution can be applied to the signal produced by TDI detector 44 to provide better resolution of the corresponding FISH spots that are illustrated.

Again, to avoid any confusion, those skilled in the art will appreciate that in the present invention, which is employed for the analysis of reporter labeled beads, a bead in analogous to the cell and a reporter is analogous to a FISH spot, in regard to the discussion of FISH spots contained herein. One difference is that a FISH spot is typically constructed with a single fluorochrome and therefore emits over a single

spectral range, as will be evident in the Figures. In the case of reporters, each reporter may be constructed of more than one fluorochrome and therefore, can emit several spectra simultaneously. The same signal processing techniques disclosed in the above-referenced U.S. Patent No. 6,211,955 and discussed relative to objects and FISH spots can be applied to enumerate unique reporters of more than one fluorochrome associated with, or bound to a bead. Therefore, it will be understood that the imaging system and techniques used for analysis may be used to enumerate unique reporters associated with or bound to a bead in order identify the unique spectral signature of the bead.

FIGURE 17 illustrates the results of processing the images on the detector in a reporter labeled bead scenario, wherein the emission spectra from each reporter has been deconvolved. FIGURE 17, which is discussed in greater detail below, is also used to illustrate the use of the non-convolving spectral decomposition and imaging system discussed in the next section.

Second and Third Embodiments of Apparatus for Spectral Decomposition and Imaging

In the second embodiment of apparatus usable in practicing the present invention, a spectral dispersing component having characteristics that ensure no distortion or convolution of the image occurs due to the emission bandwidth is employed, and as a result, a deconvolution step is not needed to process the image data. FIGURE 11 illustrates this second preferred embodiment. Unlike a prism, where every wavelength leaves the prism at a different angle, all light within a predefined bandwidth incident on the dichroic beam splitter at a common angle leaves the dichroic beam splitter at the same angle. Consequently, there is no convolution between the emission spectrum of the light leaving the bead and the image of that bead. When using such a spectral dispersing component, light of a first spectral bandwidth is reflected from the first dichroic beam splitter at a predefined nominal angle. Light of a second spectral bandwidth is passed through the first dichroic beam splitter to the next dichroic beam splitter and is reflected therefrom at a different predefined nominal angle. Light of a third spectral bandwidth is passed through the first and second dichroic beam splitters to a third dichroic beam splitter and reflected therefrom at a third predefined nominal angle. The dichroic beam splitters are selected to cover the desired light spectrum with the appropriate spectral passbands. In FIGURE 11 illustrates a five-color stacked wedge spectral dispersing filter assembly 252. This second embodiment is substantially similar to the embodiment shown in FIGURES 2 and 3, except that spectral dispersing prism element 36 (of FIGURES 2 and 3) is replaced by spectral dispersing filter assembly 252. The spectral dispersing filter assembly splits the light into a plurality of light beams having different bandwidths. Each light beam thus

produced is directed at a different nominal angle so as to fall upon a different region of detector 44. The nominal angular separation between each bandwidth produced by the spectral dispersing filter assembly 252 exceeds the field angle of the imaging system in object space thereby preventing overlap of the field images of various bandwidths on the detector.

Spectral dispersing filter assembly 252 comprises a plurality of stacked dichroic wedge filters, including a red dichroic filter R, an orange dichroic filter O, a yellow dichroic filter Y, a green dichroic filter G, and a blue dichroic filter B. Red dichroic filter R is placed in the path of collected light 34, oriented at an angle of approximately 44.0° relative to an optic axis 253 of collection lenses 32 and 32. Light of red wavelengths and above, i.e., > 640 nm, is reflected from the surface of red dichroic filter R at a nominal angle of 1°, measured counter-clockwise from a vertical optic axis 257. Example spectral reflectance characteristics R' of red dichroic filter R are plotted in FIGURE 12, along with example spectral reflectance characteristics corresponding to the other dichroic filters used in spectral dispersing filter assembly 252. In FIGURE 12, O' indicates the spectral reflectance characteristics of orange dichroic filter O, Y' indicates the spectral reflectance characteristics of yellow dichroic filter Y, etc. The light reflected by red dichroic filter R leaves spectral dispersing filter assembly 252 and passes through imaging lenses 40a and 40b, which cause the light to be imaged onto a red light receiving region of TDI detector 44, which is disposed toward the right end of the TDI detector, as shown in FIGURE 11.

Orange dichroic filter O is disposed a short distance behind red dichroic filter R and is oriented at an angle of 44.5 degrees with respect to optic axis 253. Light of orange wavelengths and greater, i.e., > 610 nm, is reflected by orange dichroic filter O at a nominal angle of 0.5° with respect to vertical optic axis 257. Because the portion of collected light 34 comprising wavelengths longer than 640 nm was already reflected by red dichroic filter R, the light reflected from the surface of orange dichroic filter O is effectively bandpassed in the orange colored region between 610 nm and 640 nm. This light travels at a nominal angle of 0.5° from vertical optic axis 257, and is imaged by imaging lenses 40a and 40b so as to fall onto an orange light receiving region disposed toward the right-hand side of TDI detector 44 between a center region of the TDI detector and the red light receiving region.

Yellow dichroic filter Y is disposed a short distance behind orange dichroic filter O and is oriented at an angle of 45° with respect to optic axis 253. Light of yellow wavelengths, i.e., 560 nm and longer, is reflected from yellow dichroic filter Y at a nominal angle of 0.0° with respect to vertical optic axis 257. Wavelengths of light reflected by yellow dichroic filter Y are effectively bandpassed in the yellow region